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# A pleiotropic missense variant in *SLC39A8* is associated with Crohn's disease and human gut microbiome composition

**Short Title: *SLC39A8* associated with CD and gut microbiome**

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**Abbreviations used in this paper:** Ala, alanine; BMI, body mass index; CI, confidence intervals; GWAS, genome-wide association studies; LD, linkage disequilibrium; MLI, Mucosal Luminal Interface; NIH, National Institutes of Health; OR, odds ratio; OTU, operational taxonomic unit; PRISM, Prospective Registry in IBD Study at Massachusetts General Hospital; SNP, single nucleotide polymorphism; Thr, threonine.

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management. All authors saw, had the opportunity to comment on, and approved the final draft.



## Abstract

**BACKGROUND & AIMS:** Genome-wide association studies (GWAS) have identified 200 inflammatory bowel disease (IBD) loci, but the genetic architecture of Crohn's disease (CD) and ulcerative colitis (UC) remains incompletely defined. Here we aimed to identify novel associations between IBD and functional genetic variants using the Illumina ExomeChip.

**METHODS:** Genotyping was performed in 10,523 IBD cases and 5,726 non-IBD controls. 91,713 functional single nucleotide polymorphism (SNP) loci in coding regions were analyzed. A novel identified association was further replicated in two independent cohorts. We further examined the association of the identified SNP with microbiota from 338 mucosal lavage samples in the Mucosal Luminal Interface (MLI) cohort measured using 16S sequencing.

**RESULTS:** We identified an association between CD and a missense variant encoding alanine (Ala) or threonine (Thr) at position 391 in the zinc transporter solute carrier family 39, member 8 protein (SLC39A8 Ala391Thr, rs13107325) and replicated the association with CD in two replication cohorts (combined meta-analysis  $p=5.55 \times 10^{-13}$ ). This variant has previously been associated with distinct phenotypes including obesity, lipid levels, blood pressure and schizophrenia. We subsequently determined that the CD-risk allele was associated with altered colonic mucosal microbiome composition in both healthy controls ( $p=0.009$ ) and CD cases ( $p=0.0009$ ). Moreover, microbes depleted in healthy carriers strongly overlap with those reduced in CD patients ( $p=9.24 \times 10^{-16}$ ) and overweight individuals ( $p=6.73 \times 10^{-16}$ ).

**CONCLUSIONS:** Our results suggest that an *SLC39A8*-dependent shift in the gut microbiome could explain its pleiotropic effects on multiple complex diseases including CD.

**Keywords:** Genetics; Inflammatory Bowel Diseases; Microbiota

## Introduction

The inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing inflammatory conditions of the gastrointestinal tract.<sup>1</sup> These diseases are a significant cause of morbidity and have estimated direct and indirect costs of \$6 billion annually in the United States.<sup>2</sup>

Currently, the etiology and pathogenesis of IBD are not fully understood, but it is widely accepted that genetic factors play an important role. Common variant genome-wide association studies have identified 200 IBD-associated loci.<sup>3,4</sup> However, these loci explain only part of the variance and genetic architecture of CD and UC.<sup>3,4</sup>

Changes in the gut microbiota have also been associated with IBD.<sup>5-11</sup> IBD patients have reduced bacterial diversity, and complex compositional changes in CD or UC patients include increased Enterobacteriaceae (such as *E. coli*) and Veillonellaceae, and reduced Ruminococcaceae (such as *F. prauznitzii*), *Roseburia*, and Clostridiales. Moreover, many of the known IBD susceptibility genes are associated with recognition and processing of bacteria.<sup>3,4,12-14</sup> A 'gardening' effect of known IBD genetic variants on gut microbiome has also been reported, suggesting a role of the gut microbiota in the pathogenesis of IBD.<sup>15</sup>

In this study, we aimed to identify novel associations between IBD and functional genetic variants using the Illumina ExomeChip array in a large European ancestry cohort. We also examined the microbiome shift associated with an identified novel locus to elucidate its functional role and understand how it contributes to disease pathogenesis.

## Materials and Methods

### *Overview*

A collaborative group with a shared goal of conducting cost-effective genotyping of their case samples and shared control samples using the Illumina Infinium HumanExome BeadChip was formed. The HumanExome BeadChip was designed to complement common variant genotyping arrays by enabling cost-effective genotyping of putative functional exonic variants that were selected from over 12,000 individual exome and whole-genome sequences from diverse populations. Its content includes non-synonymous variants, splice variants, and stop altering variants, observed at least two times across two or more of the sequencing datasets. It also includes: tags for previously described GWAS hits; African American vs. European and Native American vs. European ancestry informative markers; a scaffold grid of markers designed for identity by descent analyses; a random set of synonymous variants; fingerprint SNPs shared among several major genotyping platforms; mitochondrial SNPs; chromosome Y SNPs; and HLA tag SNPs. Some of the collaborating groups designed custom content that was added to the HumanExome base content to address individual project-specific aims. The resultant Illumina Infinium HumanExome+ BeadChip was used to genotype all cases and shared control samples. Written, informed consent was obtained from all study participants and the institutional ethical review committees of the participating centers approved all protocols.

The data for all samples were pooled together in order to optimize accurate genotype calling and quality control filtering. In this manuscript, we report results from our analyses

of predicted functional SNPs (missense, nonsense or splice variants) in non-Jewish European ancestry IBD case and control samples.

***Illumina Infinium HumanExome+ BeadChip genotyping and quality control***

DNA samples from 23,789 human peripheral blood or B-lymphoblastoid cell line specimens were processed using an Illumina Infinium HumanExome+ BeadChip at Cedars-Sinai Medical Center in Los Angeles, California; The Children's Hospital of Philadelphia in Philadelphia, Pennsylvania; The Feinstein Institute for Medical Research in Manhasset, New York; and the University of Pittsburgh in Pittsburgh, Pennsylvania. A single compiled genotyping project was created (GenomeStudio v2011.1) and intensity data for 21,233 samples deemed to be the highest quality samples based on preliminary genotype call rate and p10GC statistics were used to recluster all SNPs, and then the resultant cluster file was applied to all samples. Variants were then systematically reviewed based on several marker statistic parameters including cluster separation, theta mean and deviation, heterozygous excess and frequency, call frequency, minor allele frequency, R intensity mean, and replicate error rate, in addition to review of mitochondrial and Y chromosome markers and indels.<sup>16</sup> Following these quality control metrics, 6,849 SNPs were excluded without further manual review and 48,962 SNPs were manually reviewed and when possible, cluster locations adjusted to achieve optimal allele-calling. There was 99.9963% concordance for genotypes in 273 replicate control samples.

After genotype calling was complete, 1,161 samples were excluded based on the following criteria: p10GC and call rate statistics, gender discrepancies between reported and genotype-determined gender or ambiguous genotype-determined gender, misidentified

samples, outlier samples consistently clustering outside the three distinct genotype clusters as identified by manual review of intensity data plots, high heterozygosity, and genetic relatedness. After the genotype calling and quality control filtering steps, data for 207,625 polymorphic SNP assays in 22,628 individuals remained.

We focused our subsequent analyses on 10,523 IBD cases (5,742 CD, 4,583 UC and 198 IBD unclassified) and 5,726 controls that formed a major European ancestry cluster based on principal components analyses, and on 153,486 autosomal and chromosome X SNPs predicted to be functional (missense, nonsense or splice variants) and available in the HumanExome base content with  $\leq 0.5\%$  missing data and Hardy-Weinberg equilibrium p-value in controls  $\geq 1 \times 10^{-5}$ .

### ***Statistical analyses***

We adopted strategies previously utilized for ExomeChip single SNP analysis<sup>17</sup>. SNPs with at least 6 copies of minor alleles observed in the sample set were included in the single SNP analysis, and 61,773 SNPs with less than 6 copies were excluded. For the 91,713 variants included in the single SNPs analysis, the significance threshold was  $5.45 \times 10^{-7}$  after Bonferroni correction. To account for the rare variants in single SNP analysis, statistical inference on trait-SNP association was performed using linear regression assuming an additive genetic model, following examples in a previous study.<sup>17</sup> In single SNP analysis, we also utilized logistic regression to estimate the Odds ratios (OR) and 95% confidence intervals (95% CI) when applicable. The first four principle components were included in the model as covariates to control for potential confounding effects due to population stratification. In addition to the standard genotyping quality control measures (listed

above), genotype clusters for key SNPs listed in main tables were manually reviewed by two independent research personnel to ensure accurate allele-calling.

### ***Replication cohorts***

To validate novel association findings, we used two additional cohorts, including non-overlapping samples from a pediatric IBD GWAS cohort (1,096 CD cases and 6,088 non-IBD controls)<sup>18</sup> and the Prospective Registry in IBD Study at Massachusetts General Hospital (PRISM) exome chip cohort (551 CD cases and 2,344 non-IBD controls).<sup>19</sup> In both cohorts, association was tested using logistic regression with adjustment for principal components. We also performed an inverse-variance meta-analysis to combine results from all three cohorts, leading to a total sample size of 7,389 cases and 14,158 controls.

### ***Microbiome analysis***

The MLI cohort consists of 338 mucosal lavage samples from the cecum and sigmoid colon (i.e. 2 samples per person) of healthy individuals (22 SLC39A8 Thr391 allele carriers and 75 non-carriers) and CD patients in endoscopic remission (16 SLC39A8 Thr391 allele carriers and 58 non-carriers).<sup>20</sup> Genomic DNA extraction, V4 region 16S ribosomal RNA gene amplification, data-preprocessing, and 97% operational taxonomic unit (OTU) picking were performed as previously described,<sup>20</sup> yielding a median sampling sequencing depth of 606,105. Alpha diversity was assessed using the number of observed species, Chao1, phylogenetic diversity, and Shannon index on rarefied data. 16S rRNA abundances underwent normalization by a scaling factor (median of ratios of OTU counts to geometric mean across all samples).<sup>21</sup> Distance matrices were calculated using root square Jensen-

Shannon divergence, and then principal coordinates analysis was performed in QIIME. Additional beta diversity metrics including unweighted UniFrac, weighted UniFrac, and Bray–Curtis were measured using rarefied data in QIIME. P-values were calculated using Adonis.

Analysis of association between novel IBD-associated genetic variants and OTUs or genera was performed using Phyloseq<sup>22</sup> and the DESeq2 algorithm (<http://www.bioconductor.org/packages/release/bioc/html/DESeq2.html>).<sup>23</sup> OTUs present in less than 10% of samples were removed prior to analysis. An empirical Bayesian approach was used to shrink dispersion of normalized count data. Log fold changes for each OTU were fitted to a general linear model (fixed effects only) under a negative binomial model. Multivariate models included gender, lavage site, disease status, body mass index (BMI) ( $25 < \text{or} > 25$ ), and *SLC39A8* carrier status. OTUs or genera were filtered out by choosing a mean count threshold maximizing the number of OTUs returned at a given false discovery rate. Outliers were replaced by trimmed means, and p-values for the coefficients for carrier status in the linear models were calculated using the Wald test, then converted to q-values (<http://www.bioconductor.org/packages/release/bioc/html/qvalue.html>). Associations were considered significant if they were below a q-value threshold of 0.05. Hypothesis inference on the overlap of OTUs associated with CD, obesity and the *SLC39A8* Thr391 allele was performed using the log-linear model.

## Results

We analyzed 91,713 rare and common functional (missense, nonsense or splice variant) polymorphic SNPs that passed quality control (Table S1). Complete results for all SNPs in the single SNP analysis can be found in Table S2. QQ plots show modest genomic inflation ( $\lambda_{GC}$ =1.074, 1.093 and 1.094 for CD, UC and IBD, respectively). Functional variants in previously reported IBD loci such as *NOD2*, *IL23R*, and *CARD9*<sup>3,4</sup> were significantly associated with CD, UC or both forms of IBD after Bonferroni correction ( $p < 5.45 \times 10^{-7}$ ) (Table S3). Furthermore, an additional common missense variant, rs13107325, which encodes SLC39A8 Ala391Thr, was significantly associated with CD ( $p = 3.77 \times 10^{-8}$ ; OR=1.31, 95% CI 1.19-1.44) (Table 1). We also examined the effects of different genotypes of the Ala391Thr variant and observed an OR of 1.32, 95% CI 1.19-1.47, for the heterozygous Ala/Thr genotype (genotype frequency 16.64% in CD and 13.10% in controls,  $p = 1.66 \times 10^{-7}$ ) and an OR of 1.53, 95% CI 0.96-2.36, for the homozygous Thr/Thr genotype (genotype frequency 0.92% in CD and 0.63% in controls,  $p = 0.054$ ), suggesting an additive heredity model. We checked the conservation of the SLC39A8 Ala391Thr variant across different species and observed a PolyPhen<sup>24</sup> score of 0.782, indicating that this variant is “possibly damaging.” SLC39A8 Ala391Thr has a PHRED score of 18.44 in the Combined Annotation Dependent Depletion (CADD) database,<sup>25</sup> suggesting that it is among the top 1.43% most deleterious substitutions possible in the human genome.

*SLC39A8* is located approximately 200 Kb centromeric from *NFKB1*, a gene in a known UC-associated locus, and 250 Kb downstream of *BANK1*, a gene in a known CD-associated locus.<sup>3</sup> Conditional analyses were performed by including both index SNPs (rs13126505 in



*BANK1* and rs1598859 in *NFKB1*, which has a linkage disequilibrium (LD)  $r^2=1$  with the reported rs3774959<sup>3</sup>) in the model (Table 1). Effect estimates for *SLC39A8* Ala391Thr did not vary much ( $OR_{cond}=1.28$ , 95% CI 1.12-1.47), although the p-value dropped to  $3.00 \times 10^{-4}$  conditional on the two index variants in *NFKB1* and *BANK1*. Interestingly, the effect of the known CD-associated *BANK1* SNP rs13126505, which is in moderate LD with the SNP encoding *SLC39A8* Ala391Thr ( $r^2=0.50$ ), greatly diminished in the joint model (in single SNP analysis:  $p=4.43 \times 10^{-5}$ ,  $OR=1.24$ ; in joint model:  $p=0.021$ ,  $OR=1.07$ ). Furthermore, the *SLC39A8* SNP remained associated with CD in an analysis that was stratified by the *BANK1* SNP (rs13126505), but the *BANK1* SNP was no longer significant in the reciprocal stratification analysis (Table S4), indicating that the previously identified association signal at the *BANK1* locus<sup>3</sup> is at least partially driven by the *SLC39A8* missense variant. The UC-associated *NFKB1* SNP is not in LD with the SNP encoding the CD-associated *SLC39A8* Ala391Thr variant ( $r^2=0.004$ ), strongly suggesting that its effect is independent of the observed association with *SLC39A8*. We also examined the interaction between *SLC39A8* Ala391Thr and known CD SNPs genotyped on the HumanExome+ BeadChip and observed no statistically significant interactions after controlling for multiple testing (Table S5).

We then replicated the *SLC39A8* Ala391Thr association in two independent cohorts (Table 1). We observed similar association in both replication cohorts (pediatric CD GWAS cohort:  $p=2.35 \times 10^{-3}$ ;  $OR=1.26$ , 95% CI 1.09-1.46; PRISM cohort:  $p=1.20 \times 10^{-4}$ ;  $OR=1.56$ , 95% CI 1.24-1.95). The evidence for association between CD and *SLC39A8* Ala391Thr was highly significant ( $p=5.55 \times 10^{-13}$ ;  $OR=1.33$ , 95% CI 1.21-1.45) in a meta-analysis of the combined discovery and replication cohorts.

A genotype-subphenotype correlation analysis (Table 2) for *SLC39A8* Ala391Thr found significant evidence for association with ileal CD ( $p=0.002$  compared to non-ileal CD; OR=1.36, 95% CI 1.12-1.66) and with complicated disease behavior ( $p=0.006$  for stricturing (B2) or penetrating (B3) vs. non-stricturing, non-penetrating (B1) behavior; OR=1.22, 95% CI 1.06-1.40). Joint analysis indicates that the associations of *SLC39A8* with ileal CD and B2/B3 are independent signals (data not shown).

Recognizing the role that zinc plays in innate immunity and the pleiotropic nature of this polymorphism with association with other microbiome associated traits including obesity, we hypothesized that the *SLC39A8* genetic variant may affect CD susceptibility by altering the microbiome. Therefore, we tested the microbial composition of lavage samples from the cecum and sigmoid colon of *SLC39A8* Thr391 allele carriers and non-carriers in the MLI<sup>15, 20, 26</sup> cohort. CD patients and healthy controls were analyzed separately and were further subdivided by BMI (greater or less than 25) given previous associations of both CD and obesity with the microbiome. Carriage of the CD-associated Thr allele was associated with altered microbial composition as determined by 16S ribosomal RNA sequencing in both healthy controls and CD patients with BMI<25 ( $p=0.009$  and  $0.0009$ , respectively) (Figure 1A). We focused on carrier-associated changes in the microbiome of non-overweight healthy controls to capture pre-disease perturbations that may predispose to CD and obesity. A multivariate analysis of the non-overweight healthy controls incorporating carrier status, sampling site, and gender revealed statistically significant enrichment of 54 OTUs and depletion of 823 OTUs in the Thr allele carriers (Table S6). We further investigated whether the microbial signature of non-overweight healthy *SLC39A8* variant carriers resembled that of CD patients. Indeed, 290 of the 823 depleted OTUs

reduced in healthy Thr allele carriers were also reduced in non-carrier, non-overweight CD patients compared to non-carrier, non-overweight healthy controls, indicating a strongly statistically significant overlap ( $p=9.24 \times 10^{-16}$ ) of the shift in gut microbiota (Figure 1B, Table S7). There was similar overlap of the *SLC39A8* microbial signature with that of individuals with BMI>25: 443 OTUs depleted in non-overweight healthy Thr allele carriers were also reduced in overweight, non-IBD, non-carriers ( $p=6.73 \times 10^{-16}$ ) (Table S8). This overlap was not observed in the enriched OTUs (19 and 13 of the 54 enriched OTUs also associated with CD and overweight status, with  $p=0.88$  and  $0.10$ , respectively).

Among relatively abundant bacterial taxa, five genera depleted in *SLC39A8* Thr391 allele carriers were also significantly ( $q<0.05$ ) depleted in CD patients and overweight controls (*Anaerostipes*, *Coprococcus*, *Roseburia*, *Lachnospira*, and *SMB53*) (Figure 1C). Additional genera depleted in carriers and CD included *Faecalibacterium prausnitzii* and *Ruminococcus gnavus*. The most strongly depleted genus in Thr allele carriers was [*Eubacterium*] ( $q=9 \times 10^{-10}$ ), attributable to reduced abundance of three OTUs identified as *E. bifforme* (Figure 1C). Other changes specific to Thr allele carriers included specific species of the *Bacteroides* (e.g., *B. eggerthii* and *B. fragilis*), *Dorea* genera *Sutterella*, and *Collinsella aerofaciens*.

## Discussion

In the current study, we utilized the Illumina ExomeChip to identify functional variants associated with IBD. We found that a missense variant in *SLC39A8*, a pleiotropic locus with effects in several phenotypes including hypertension, blood lipid, obesity as well as schizophrenia, is associated with CD. We replicated this finding in two independent cohorts and illustrated that this locus is also associated with disease location and behavior in CD. We further demonstrated that this locus is associated with shifts in the composition of the gut microbiota in both CD and controls. Moreover, these *SLC39A8*-associated shifts in the composition of the gut microbiota strongly overlap with gut microbiota changes associated with CD and obesity.

*SLC39A8* is part of a family of zinc transporters that is localized to the plasma membrane and early endosomes<sup>27</sup> and mediates zinc influx into the cytosol.<sup>28, 29</sup> Zinc has concentration-dependent effects on immune function, and zinc deficiency has been shown to affect T cell counts and function.<sup>30</sup> *SLC39A8* is highly expressed in T cells, stimulated monocytes, and differentiated macrophages,<sup>28, 31</sup> and a recent study demonstrated that continuous stimulation of pattern recognition receptors in macrophage derived monocytes increased intracellular zinc concentrations and enhanced macrophage clearance of bacteria via autophagy.<sup>32</sup> Upregulated expression of *SLC39A8* during T cell activation leads to increased intracellular zinc in the cytoplasm and increased IFN- $\gamma$  expression.<sup>28</sup> In addition, *SLC39A8* is upregulated in response to tumor necrosis factor-alpha and lipopolysaccharide and serves as a negative regulator of innate immune function and of NF- $\kappa$ B signaling via zinc-mediated suppression of I $\kappa$ B kinase in monocytes and macrophages.<sup>33, 34</sup> Such effects

on T cell function and innate immunity are of potential key pathophysiologic relevance to IBD, and our findings highlight the importance of further defining the role of zinc in IBD. In a study of treatment naïve pediatric IBD patients, *SLC39A8* was one of 1,281 genes that were differentially expressed in ileal biopsies from patients with CD.<sup>35</sup> *SLC39A8* Ala391Thr has also been associated with lipid levels, blood pressure, obesity and schizophrenia, emphasizing the pleiotropic and critical role of this variant in health and disease as well as further highlighting the intriguing relationship among metabolic features, psychiatric illness, and chronic inflammation.<sup>36-41</sup>

CD, dyslipidemia and obesity have all been linked to alterations in the intestinal microbiome,<sup>6, 42</sup> and a recent study linked the same gut microbiome change (decrease in *Akkermansia muciniphila*) to both IBD and type II diabetes.<sup>43</sup> Furthermore, in a study of the microbiome in new-onset CD, an exploratory analysis of genomes of organisms associated with CD identified a contribution to pathway components that included a zinc-dependent enzyme.<sup>6</sup> We therefore hypothesized that the *SLC39A8* genetic variant identified in this study may affect CD susceptibility by altering the microbiome. In the microbiome analysis, 5 genera (*Anaerostipes*, *Coprococcus*, *Roseburia*, *Lachnospira*, and *SMB53*) are depleted in *SLC39A8* Thr391 allele carriers, CD patients and overweight controls. *Faecalibacterium prausnitzii* and *Ruminococcus gnavus* are depleted in both Thr allele carriers and CD. These genera (with the exception of *SMB53*) are notable for being producers of short-chain fatty acids, which can ameliorate colitis through induction of regulatory T cells.<sup>44, 45</sup> Prior studies also found lower levels of *Faecalibacterium prausnitzii* in IBD,<sup>6, 11, 46, 47</sup> and higher levels of this bacterium are associated with lower risk of postoperative CD recurrence and maintenance of remission in UC.<sup>47, 48</sup> An early small Sanger sequencing study of ileal

biopsies had suggested increased *Ruminococcus gnavus* in CD,<sup>49</sup> possibly reflecting differences in ileal vs. colonic location or differences among *Ruminococcus gnavus* strains in their association with CD. This organism was also depleted in CD patients compared to healthy controls, an association not previously reported but perhaps related to the unique mucosal lavage sampling protocol used for this cohort. Thus, *SLC39A8* is part of an emerging set of loci associated with taxonomically-restricted microbial composition.<sup>48</sup>

We investigated the effect of *SLC39A8* Ala391Thr on protein structure. The closest transporters to *SLC39A8* are *SLC39A14* and *SLC39A12* with 50% sequence identity. There is no known X-ray 3D structure for *SLC39A8*, and since this region is not homologous to other zinc transporters with X-ray 3D structures, the effect of the Ala391Thr variant on the structure of *SLC39A8* cannot be inferred. Thus the effect of this variant on transporter structure is uncertain. It is notable that the Ala391Thr variant is located in a transmembrane domain (based on Uniprot) and mutations involving polar residues are particularly associated with protein malfunction.<sup>50</sup> Loss of function of *SLC39A8*, a zinc solute transporter, may perturb zinc uptake by epithelial and immune cells and cause zinc deficiency as has been observed in *SLC39A8* hypomorphic mice.<sup>51</sup> Animal studies have indicated that zinc deficiency alters microbial function and composition.<sup>52</sup> Moreover, some ion channels, such as *KCNN4*, which share membrane transporter properties and roles with solute transporters, are activated by intracellular calcium and regulate Paneth cell secretion<sup>53</sup> with an impact on immune cell and epithelial cell function.<sup>54</sup> Therefore, we speculate that by disturbing the transmembrane domain of *SLC39A8*, Ala391Thr may alter zinc metabolism in functionally relevant cells, which might in turn affect innate and adaptive immunity, as well as the gut microbiota.

In summary, we identified and replicated a novel association between CD and *SLC39A8*, a zinc transporter linked to multiple metabolic traits. The *SLC39A8* Ala391Thr variant identified in our study was associated with altered microbiome composition in both healthy controls and CD patients. Moreover, the observed microbe signature in healthy *SLC39A8* Thr391 allele carriers strongly overlaps with that in CD patients and overweight individuals, suggesting a pre-disease microbial susceptibility state originating due to genetic influence on the microbial ecosystem that contributes to the pleiotropic effect of *SLC39A8* in CD and metabolic traits.

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**Figure 1. *SLC39A8* polymorphism is associated with shifts in the colonic microbiome paralleling those of Crohn's disease and obesity.** A) Principal coordinates analysis plots of the MLI cohort, divided into subsets by CD status and BMI. Individuals are colored by *SLC39A8* Thr391 allele carrier status. Each PCoA plot shows the number of samples from carriers (red) and non-carriers (blue) in the upper left corner and p-values for the difference in microbial composition between carriers and non-carriers in the upper right corner. B) Venn diagrams depicting the overlap of enriched and depleted OTUs in three comparisons: *SLC39A8* Thr391 allele carriers vs. non-carriers in the non-CD subset with BMI<25, CD vs. non-CD individuals in the non-carrier subset with BMI<25, and BMI>25 vs. BMI<25 in the non-carrier, non-CD subset. OTUs were identified as enriched or depleted if they had  $q < 0.05$  in multivariate models with sample site (cecum, sigmoid) and gender as additional covariates. C) Log2 fold changes (FC) for OTUs with a statistically significant difference in abundance between carriers and non-carriers from multivariate models. Only OTUs identified to the genus level with mean normalized abundance  $> 10^{-4}$  in the non-IBD, BMI<25 subset are shown. Colors correspond to the Venn diagrams in panel B. Genera are ordered by the log2 fold changes of entire genera between carriers and non-carriers.

**Table 1. Meta-analysis of the association of SLC39A8 Ala391Thr variant with CD in discovery and replication cohorts**

Gene	SNP	Cohort	N cases	N controls	FA*	FU^	P	OR(95% CI)
<b>SLC39A8</b>	Ala391Thr	Discovery cohort	5742	5726	0.092	0.072	3.77x10 <sup>-8</sup>	1.31(1.19-1.44)
		Pediatric CD GWAS	1096	6088	0.108	0.088	2.35x10 <sup>-3</sup>	1.26(1.09-1.46)
		PRISM	551	2344	0.109	0.072	1.20x10 <sup>-4</sup>	1.56(1.24-1.95)
		Meta-analysis	7389	14158	-	-	5.55x10 <sup>-13</sup>	1.33(1.21-1.45)

\*FA: Frequencies of Thr allele in CD

^FU: Frequencies of Thr allele in non-IBD



**Table 2. Association of SLC39A8 Ala391Thr with clinical phenotypes of CD**

Gene	SNP	Outcome*	N	P	OR(95% CI)
SLC39A8	Ala391Thr	B2 vs. B1	3415	0.030	1.20(1.02-1.42)
		B3 vs. B1	3602	0.012	1.23(1.05-1.45)
		B3 or B2 vs. B1	4852	0.006	1.22(1.06-1.40)
		Colonic location	4942	0.966	1.00(0.86-1.17)
		Ileal location	4823	0.002	1.36(1.12-1.66)
		Jejunal location	4460	0.205	1.17(0.92-1.50)
		Perianal disease	4876	0.868	0.99(0.85-1.15)
		Surgery	4841	0.268	1.08(0.94-1.24)

\* Disease behavior classified according to Montreal classification: B1 – inflammatory or non-stricturing/non-internal penetrating phenotype; B2 – Stricturing phenotype; B3 – internal penetrating or fistulizing phenotype.

Surgery is defined as abdominal surgery for complication of Crohn's disease.

